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Specification and Drawings, as originally filed, with Application for Patent Serial No: **2,397,379**, on August 9, 2002, by **OTTAWA HEALTH RESEARCH INSTITUTE**, assignee of May Griffith, David J. Carlsson and Fengfu Li, for "Bio-Synthetic Matrix and Uses Thereof".

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BIO-SYNTHETIC MATRIX AND USES THEREOF

ABSTRACT

The present invention provides a bio-synthetic matrix comprising a hydrogel which is formed by cross-linking a synthetic polymer and a bio-polymer. The matrix is robust, biocompatible and non-cytotoxic, can be formed at neutral pH in water and is capable of supporting cell in-growth *in vivo*. The matrix can be tailored to further comprise one or more bioactive agents. The matrix may also comprise cells encapsulated and dispersed therein, which are capable of proliferating upon implantation of the matrix *in vivo*. The present invention also provides a method of preparing the bio-synthetic matrix and for the use of the matrix *in vivo* for tissue engineering or drug delivery applications.

FIELD OF THE INVENTION

The present invention pertains to the field of tissue engineering and in particular to a bio-synthetic matrix comprising a hydrogel suitable for implantation *in vivo*.

BACKGROUND

Tissue engineering is a rapidly growing field encompassing a number of technologies aimed at replacing or restoring tissue and organ function. The key objective in tissue engineering is the regeneration of a defective tissue through the use of materials that can integrate into the existing tissue so as to restore normal tissue function. Tissue engineering, therefore, demands materials that can support cell in-growth or encapsulation and, in many cases, nerve regeneration.

Polymer compositions are finding widespread application in tissue engineering. Natural bio-polymers such as collagens, fibrin, alginates and agarose are known to be non-cytotoxic and to support in-growth and encapsulation of living cells. Matrices derived from natural polymers, however, are generally insufficiently robust for transplantation. In contrast, matrices prepared from synthetic polymers can be formulated to exhibit predetermined physical characteristics such as gel strength, as well as biological characteristics such as degradability. Reports that synthetic analogues of natural polymers, such as polylysine, polyethylene imine, and the like, can exhibit cytotoxic effects [Lynn & Langer, *J. Amer. Chem. Soc.*, 122:10761-10768 (2000] have lead to the development of alternative synthetic polymers for tissue engineering applications.

Hydrogels are crosslinked, water-insoluble, water-containing polymers which offer good biocompatibility and have a decreased tendency to induce thrombosis, encrustation, and inflammation and as such are ideal candidates for tissue engineering purposes. The use of hydrogels in cell biology is well known [see for example A. Atala and R.P. Lanza, eds., "Methods in Tissue Engineering" Academic Press, San Diego, 2002]. A wide variety of hydrogels for *in vivo* applications have been described [see, for example, the review by Jeong, *et al.*, *Adv. Drug Deliv. Rev.*, 54:37-51 (2002)]. Hydrogels based on N-isopropylacrylamide (NiPAAm) and certain co-polymers thereof, for example, are non-toxic and capable of

supporting growth of encapsulated cells *in vitro* [Vernon, *et al., Macromol. Symp.*, 109:155-167 (1996); Stile, *et al., Macromolecules*, 32:7370-9 (1999); Stile, *et al., Biomacromolecules* 3: *591 – 600.(2002);* Stile, *et al., Biomacromolecules* 2: *185 – 194.(2001);* Webb, *et al., MUSC Orthopaedic J.*, 3:18-21 (2000); An *et al.*, U.S. Patent No. 6,103,528]. However, despite manipulations of synthesis conditions and improvements to enhance biocompatibility, it is still difficult to obtain a seamless host-implant interface and complete integration of the implant into the host [Hicks, et al. Surv. Ophthalmol. 42: 175-189 (1997); Trinkaus-Randall and Nugent, J. Controlled Release 53:205-214 (1998)].

Modifications of synthetic polymer gels with a second naturally derived polymer to generate an interpenetrating polymer network ("IPN") structure have been reported [For example, see Gutowska *et al., Macromolecules*, 27:4167 (1994); Yoshida *et al., Nature*, 374:240 (1995); Wu & Jiang, U.S. Patent No. 6,030,634; Park *et al.*, U.S. Patent No. 6,271,278]. However, these structures are frequently destabilised by extraction of the naturally derived component by culture media and by physiological fluids. Naturally derived polymers also tend to biodegrade rapidly within the body resulting in destabilisation of *in vivo* implants.

More robust hydrogels comprising cross-linked polymer compositions have also been described. For example, U.S. Patent No. 6,388,047 describes a composition consisting of a hydrophobic macromer and a hydrophilic polymer that are cross-linked to form a hydrogel by free-radical polymerisation. U.S. Patent No. 6,323,278 describes a cross-linked polymer composition which can form *in situ* and which comprises two synthetic polymers, containing multiple electrophilic groups and the other containing multiple nucleophilic groups. Both U.S. Patent No. 6,388,047 and 6,384,105 describe systems that must be cross-linked by free radical chemistry, which requires the use of initiators that are well known to be cytotoxic (azo compounds, persulfates), thus leading to possible side effects if the hydrogel was to be used in the tissue or with encapsulated cells.

U.S. Patent No. 6,384,105 describes injectable, biodegradable polymer composites comprising poly(propylene fumarate) and poly(ethylene glycol)-dimethacrylate which can be cross-linked *in situ*. The hydrogels described in this patent are largely based on polymers with a polyethylene

oxide backbone polymers. Although these polymers are known to be biocompatible, their ability to support cell growth is uncertain.

There remains a need therefore, for a matrix that is biocompatible, sufficiently robust to function as an implant and that supports cell growth *in vivo*.

This background information is provided for the purpose of making known information believed by the applicant to be of possible relevance to the present invention. No admission is necessarily intended, nor should be construed, that any of the preceding information constitutes prior art against the present invention.

SUMMARY OF THE INVENTION

An object of the present invention is to provide a bio-synthetic matrix and uses thereof. In accordance with an aspect of the present invention, there is provided a bio-synthetic matrix comprising a hydrogel formed by cross-linking a synthetic polymer and a bio-polymer, wherein said synthetic polymer comprises one or more acrylamide derivatives, one or more hydrophilic co-monomers and one or more derivatized carboxylic acid co-monomers comprising pendant cross-linking moieties.

In accordance with another aspect of the invention, there is provided a use of the bio-synthetic matrix as a scaffold to support tissue repair and regeneration *in vivo*.

In accordance with another aspect of the invention, there is provided a use of the bio-synthetic matrix to deliver one or more bioactive agents into a tissue or organ of a mammal.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts the general structure of the terpolymer of N-isopropylacrylamide, (NiPAAm), acrylic acid (AAc) and N-acryloxysuccinimide (ASI).

Figure 2 depicts the transplantation into pigs of artificial corneas prepared from a bio-synthetic matrix prepared by cross-linking collagen and a terpolymer of N-isopropylacrylamide, (NiPAAm), acrylic acid (AAc) and N-acryloxysuccinimide (ASI) with the cell adhesion motif (YSIGR) covalently bound to the terpolymer.

Figure 3 presents the clinical results from the transplantation into pigs of artificial corneas prepared from a bio-synthetic matrix prepared by cross-linking collagen and a terpolymer of N-isopropylacrylamide, (NiPAAm), acrylic acid (AAc) and N-acryloxysuccinimide (ASI) with the cell adhesion motif (YSIGR) covalently bound to the terpolymer.

Figure 4 depicts *in vivo* confocal microscopy of artificial corneas transplanted into pigs. Artificial corneas were prepared from a bio-synthetic matrix prepared by cross-linking collagen and a terpolymer of N-isopropylacrylamide, acrylic acid and N-acryloxysuccinimide with the cell adhesion motif (YSIGR) covalently bound to the terpolymer..

Figure 5 presents the results of *in vivo* confocal microscopy of artificial corneas transplanted into pigs. Artificial corneas were prepared from a bio-synthetic matrix prepared by cross-linking collagen and a terpolymer of N-isopropylacrylamide, acrylic acid and N-acryloxysuccinimide with the cell adhesion motif (YSIGR) covalently bound to the terpolymer..

Figure 6 depicts *in vivo* testing for corneal sensitivity of artificial corneas transplanted into pigs. Artificial corneas were prepared from a bio-synthetic matrix prepared by cross-linking collagen and a terpolymer of N-isopropylacrylamide, acrylic acid and N-acryloxysuccinimide with the cell adhesion motif (YSIGR) covalently bound to the terpolymer..

Figures 7, 8 and 9 present the results of morphological and biochemical assessment of artificial corneas transplanted into pigs. Artificial corneas were prepared from a bio-synthetic matrix prepared by cross-linking collagen and a terpolymer of N-isopropylacrylamide, acrylic acid and N-acryloxysuccinimide with the cell adhesion motif (YSIGR) covalently bound to the terpolymer..

Figure 10 shows (A) the structure of the terpolymer, (B) corneal scaffold composed of thermogelled collagen only, (C) corneal scaffold composed of cross-linked collagen and a terpolymer of N-isopropylacrylamide, acrylic acid and N-acryloxysuccinimide with the cell adhesion motif (YSIGR) covalently bound to the terpolymer. *In vitro* nerve growth patterns within the collagen-terpolymer composite are shown in (F) and within the underlying host stroma in (G). (H) shows in-growing stromal cells.

Figures 11 and 12 depict the delivery of a hydrogel containing collagen and the terpolymer-pentatpeptide into mouse and rat brains

DETAILED DESCRIPTION OF THE INVENTION

It should be understood that this invention is not limited to the particular process steps and materials disclosed herein, but is extended to equivalents thereof as would be recognised by those ordinarily skilled in the relevant arts. It should also be understood that terminology employed herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains.

The term "hydrogel" as used herein refers to a cross-linked polymeric material which exhibits the ability to swell in water and to retain a significant portion of water within its structure without dissolving.

The term "polymer" as used herein refers to a molecule consisting of individual monomers joined together. In the context of the present invention, a polymer may comprise monomers that are joined "end-to-end" to form a linear molecule, or may comprise monomers that are joined together to form a branched structure.

The term "monomer" as used herein refers to a simple organic molecule which is capable of forming a long chain either alone or in combination with other similar organic molecules to yield a polymer.

The term "co-polymer" as used herein refers to a polymer that comprises two or more different monomers. Co-polymers can be regular, random, block or grafted. A regular co-polymer refers to a co-polymer in which the monomers repeat in a regular pattern (*e.g.* for monomers A and B, a random co-polymer may have a sequence: ABABABAB). A random co-polymer is a co-polymer in which the different monomers are arranged randomly or statistically in each individual polymer molecule (*e.g.* for monomers A and B, a random co-polymer may have a sequence: AABABBABBBAAB). In contrast, a block co-polymer is a co-polymer in which the different monomers are separated into discrete regions within each individual polymer molecule (*e.g.* for monomers A and B, a block co-polymer may have a sequence: AAABBBAAABBB). A grafted co-polymer refers to a co-polymer which is made by linking a polymer or polymers of one type to a another polymer molecule of a different composition.

The term "bio-polymer" as used herein refers to a naturally occurring polymer. Naturally occurring polymers include, but are not limited to, proteins and carbohydrates.

The term "synthetic polymer" as used herein refers to a polymer that is not naturally occurring and that is produced by chemical or recombinant synthesis.

The term "bioactive agent" as used herein refers to a molecule or compound which exerts a physiological, therapeutic or diagnostic effect *in vivo*. Bioactive agents may be organic or inorganic. Representative examples include proteins, peptides, carbohydrates, nucleic acids and fragments thereof, anti-tumour and anti-neoplastic compounds, anti-viral compounds, anti-inflammatory compounds, antibiotic compounds such as antifungal and antibacterial compounds, cholesterol lowering drugs, contrast agents for medical diagnostic imaging, enzymes, cytokines, local anaesthetics, hormones, anti-angiogenic agents, neurotransmitters, therapeutic

oligonucleotides, viral particles, vectors, growth factors, retinoids, cell adhesion factors, laminin, hormones, osteogenic factors, antibodies and antigens.

The term "biocompatible" as used herein, refers to an ability to be incorporated into a biological system, such as into a mammalian organ or tissue, without stimulating an immune and / or inflammatory response, fibrosis or other adverse tissue response.

I. BIO-SYNTHETIC MATRIX

The present invention provides a functional bio-synthetic matrix comprising a hydrogel which is formed by cross-linking a synthetic polymer and a bio-polymer. The matrix is robust, biocompatible and non-cytotoxic. The matrix according to the present invention supports cell growth, including epithelial and endothelial surface coverage (*i.e.* two dimensional, 2D, growth), three-dimensional (3D) cell in-growth and nerve in-growth. The matrix can be tailored to further comprise one or more bioactive agents such as growth factors, retinoids, cell adhesion factors, enzymes, peptides, proteins, drugs, genes, and the like. The bioactive agent can be covalently attached to the synthetic polymer, or it may be encapsulated and dispersed within the final matrix. The matrix may also comprise cells encapsulated and dispersed therein, which are capable of proliferating and/or diversification upon implantation of the matrix *in vivo*.

I.I Synthetic Polymer

In accordance with the present invention, the synthetic polymer that is incorporated into the bio-synthetic matrix is a co-polymer comprising one or more acrylamide derivatives, one or more hydrophilic co-monomers and one or more derivatised carboxylic acid co-monomers which comprise pendant cross-linkable moieties. The co-polymer may be linear or branched, regular, random or block. In accordance with the present invention, the final synthetic polymer comprises a plurality of pendant reactive moieties available for cross-linking or grafting of appropriate biomolecules. The overall hydrophilicity of the copolymer is controlled to confer water solubility at 0°C to physiological temperatures without precipitation or phase transition.

As is known in the art, most synthetic polymers have a distribution of molecular mass and various different averages of the molecular mass are often distinguished, for example, the number average molecular mass (M_n) and the weight average molecular mass (M_w). The molecular weight of a synthetic polymer is usually defined in terms of its number average molecular mass (M_n), which in turn is defined as the sum of n_iM_i divided by the sum of n_i, where n_i is the number of molecules in the distribution with mass M_i. The synthetic polymer for use in the matrix of the present invention typically has a number average molecular mass (M_n) between 5,000 and 1,000,000. In one embodiment of the present invention, the M_n of the polymer is between about 25,000 and about 80,000. In a related embodiment, the M_n of the polymer is between about 50,000 and about 60,000. In a related embodiment, the M_n of the polymer is about 56,000.

As used herein, an "acrylamide derivative" refers to a N,N'-alkyl substituted acrylamide or methacrylamide. Examples of acrylamide derivatives suitable for use in the synthetic polymer of the present invention include, but are not limited to, N-isopropylacrylamide (NiPAAm), N,N'-diethylacrylamide, N-acryloylpyrrolidine, N-ethylacrylamide, N-isopropylmethacrylamide, N,N'-diethylmethacrylamide, N-methacryloylpyrrolidine, N-ethylmethacrylamide, and combinations thereof.

A "hydrophilic co-monomer" in the context of the present invention is a hydrophilic monomer that is capable of co-polymerisation with the acrylamide derivative component of the synthetic polymer. Examples of suitable hydrophilic co-monomers are hydrophilic acryl- or methacryl-compounds such as carboxylic acids, acrylamide, methacrylamide, hydrophilic acrylamide derivatives, hydrophilic methacrylamide derivatives, hydrophilic acrylic acid esters and hydrophilic methacrylic acid esters. The carboxylic acid may be, for example, acrylic acid, methacrylic acid, or a combination thereof. Examples of hydrophilic acrylamide derivatives include, but are not limited to, N,N-diethylacrylamide, 2-[N,N-dimethylamino]ethylacrylamide, 2-[N,N-diethylamino]ethylacrylamide, N,N-diethylmethacrylamide, 2-[N,N-dimethylamino] ethylmethacrylamide, 2-[N,N-diethylamino]ethylmethacrylamide, or combinations thereof. Examples of hydrophilic acrylic esters include, but are not limited to, 2-[N,N-diethylamino] ethylacrylate, 2-[N,N-dimethylamino]ethylacrylate, 2-[N,N-diethylamino]ethylmethacrylate, 2-

[N,N-dimethylamino]ethylmethacrylate, or combinations thereof, selected to maintain aqueous solubility and freedom from phase transition under use conditions.

As used herein, a "derivatised carboxylic acid co-monomer" refers to a hydrophilic acryl- or methacryl- carboxylic acid, for example, acrylic acid, methacrylic acid, or a combination thereof, which has been chemically derivatized to contain one or more cross-linking moieties, such as succinimidyl groups. The term "succinimidyl group" is intended to encompass variations of the generic succinimidyl group, such as sulphosuccinimidyl groups. Other similar structures such as 2-(N-morpholino)ethanesulphonic acid will also be apparent to those skilled in the art. In the context of the present invention a succinimidyl group acts to increase the reactivity of the carboxylic acid group to which it is attached towards primary amines (*i.e.* $-NH_2$ groups) and thiols (*i.e.* –SH groups). Examples of suitable derivatised carboxylic acid co-monomers for use in the synthetic polymer include, but are not limited to, N-acryloxysuccinimide.

In order for the synthetic polymer to be suitably robust and thermostable, it is important that the ratio of acrylamide derivative(s) to hydrophilic co-monomer(s) is optimised. In addition, the number of derivatised carboxylic acid co-monomer(s) present in the final polymer will determine the ability of the synthetic gel to form cross-links with the bio-polymer in the bio-synthetic matrix. In accordance with the present invention, the amount of acrylamide derivative in the polymer is between 50% and 90%, the amount of hydrophilic co-monomer is between 5% and 50%, and the amount of derivatised carboxylic acid co-monomer is between 0.1% and 15%, wherein the sum of the amounts of acrylamide derivative, hydrophilic co-monomer and derivatised carboxylic acid co-monomer is 100%.

One skilled in the art will appreciate that the selection and ratio of the components in the synthetic polymer will be dependent to varying degrees on the final application of the bio-synthetic matrix. For example, for ophthalmic applications, it is important that the final matrix be clear, whereas for other tissue engineering applications, the clarity of the matrix may not be an important factor.

In one embodiment of the present invention, the synthetic polymer is a terpolymer comprising one acrylamide derivative, one hydrophilic co-monomer and one derivatised carboxylic acid co-monomer. In a related embodiment, the synthetic polymer is a terpolymer comprising NiPAAm monomer, acrylic acid (AAc) monomer and a derivatised acrylic acid monomer. In another embodiment, the synthetic polymer is a terpolymer comprising NiPAAm monomers, acrylamide (AAm) monomers and derivatised acrylic acid monomers. In a related embodiment, the derivatised acrylic acid monomer is N-acryloxysuccinimide substituted. In another related embodiment of the present invention, the terpolymer is prepared with a feed ratio that comprises NiPAAm monomer, AAc monomer and N-acryloxysuccinimide in a ratio of about 85:10:5 molar %.

1.2 Bio-polymer

Bio-polymers are naturally-occurring polymers, such as proteins and carbohydrates. In accordance with the present invention, the bio-synthetic matrix comprises a bio-polymer cross-linked to the synthetic polymer by means of the pendant cross-linking moieties in the latter. Thus, for the purposes of the present invention the bio-polymer contains one or more groups which are capable of reacting with the cross-linking moiety (*e.g.* a primary amine or a thiol). Examples of suitable bio-polymers for use in the present invention include, but are not limited to, collagens, denatured collagens (or gelatins), fibrin-fibrinogen, elastin, glycoproteins, alginate and glucosaminoglycans. One skilled in the art will appreciate that some of these bio-polymers may need to be derivatised in order to contain a suitable reactive group, for example, glucosaminoglycans need to be deacetylated or desulphated in order to possess a primary amine group. Such derivatisation can be achieved by standard techniques and is considered to be within the ordinary skills of a worker in the art.

1.3 Bioactive Agents

The synthetic polymer according to the present invention contains a plurality of pendant cross-linking moieties. It will be apparent that sufficient cross-linking of the synthetic and bio-polymers to achieve a suitably robust matrix can be achieved without reaction of all free cross-

linking groups. Excess groups may, therefore, optionally be used to attach other desirable bioactive agents to the matrix. In one embodiment of the present invention, the cross-linking groups are succinimidyl groups and suitable bioactive agents for grafting to the polymer are those which contain either primary amino or thiol groups, or which can be readily derivatised so as to contain these groups.

Bioactive agents that may be incorporated into the matrix by cross-linking to free succinimidyl groups include, for example, growth factors, retinoids, enzymes, cell adhesion factors, laminin, hormones, osteogenic factors, cytokines, antibodies, antigens, and biologically active proteins, peptides or fragments thereof.

2. METHOD OF MAKING THE BIO-SYNTHETIC MATRIX

2.1 Preparation of the Synthetic Polymer

Co-polymerization of the components for the synthetic polymer can be achieved using standard methods known in the art [for example, see A. Ravve "Principles of Polymer Chemistry", Chapter 3. Plenum Press, New York 1995]. Typically appropriate quantities of each of the monomers are dispersed in a suitable solvent in the presence of an initiator. The mixture is maintained at an appropriate temperature and the co-polymerisation reaction is allowed to proceed for a pre-determined period of time. The resulting polymer can then be purified from the mixture by conventional methods, for example, by precipitation.

The solvent for the co-polymerisation reaction may be a non-aqueous solvent if one monomer is hydrolytically sensitive or an aqueous solvent. Suitable aqueous solvents include, but are not limited to, water, buffers and salt solutions. Suitable non-aqueous solvents are typically cyclic ethers, such as dioxane, chlorinated hydrocarbons (for example, chloroform) or aromatic hydrocarbons (for example, benzene). In one embodiment of the present invention, the solvent is a non-aqueous solvent. In a related embodiment, the solvent is dioxane.

Suitable polymerisation initiators are known in the art and are usually free-radical initiators. Examples of suitable initiators include, but are not limited to, 2,2'-azobisisobutyronitrile (AIBN), other azo compounds, such as 2,2'-azobis-2-ethylpropionitrile; 2,2'-azobis-2-cyclopropylpropionitrile; 2,2'-azobiscyclohexanenitrile; 2,2'-azobiscyclooctanenitrile, and peroxide compounds, such as dibenzoyl peroxide and its substituted analogues, and persulfates, such as sodium, potassium, *etc.*

2.2 Preparation of the Hydrogel

Cross-linking of the synthetic and bio-polymers can be readily achieved by mixing appropriate amounts of each polymer at room temperature in an appropriate solvent. Typically the solvent is an aqueous solvent, such as a salt solution, buffer solution, cell culture medium, or a diluted or modified version thereof. One skilled in the art will appreciate that in order to preserve triple helix structure of polymers such as collagen without causing fibrillogenisis and / or opacification of the hydrogel the cross-linking reation should be conducted in aqueous media at neutral pH. The significant levels of amino acids in nutrient media normally used for cell culture can cause side reactions with succinimidyl groups and other cross-linking moieties, which can result in diversion of these groups from the cross-linking reaction. Use of a medium free of amino acids and other proteinacious materials can help to prevent these side reactions and, therefore, increase the number of cross-links that form between the synthetic and bio-polymers. Conducting the cross-linking reaction in aqueous solution at room or physiological temperatures allows both cross-linking and the much slower hydrolysis of any unreacted succinimidyl groups to take place to produce a PNiPAAm-co AAc cross-linked biopolymer gel. If necessary, after the cross-linking step, the temperature of the cross-linked polymer suspension can be raised to allow the hydrogel to form.

One skilled in the art will understand that the amount of each polymer to be included in the hydrogel will be dependent on the choice of polymers and the intended application for the hydrogel. In general using higher initial amounts of each polymer will result in the formation of a more robust gel due to the lower water content and the presence of a greater amount of cross-linked polymer. In accordance with the present invention, the final hydrogel comprises between

70 and 99.7 % by weight of water, between 0.1 and 10 % by weight of synthetic polymer and between 0.3 and 30 % by weight of bio-polymer. In one embodiment of the present invention, the final hydrogel contains about 95 % by weight of water. In a related embodiment, the final hydrogel contains between about 1 – 2 % by weight of synthetic polymer and about 2 – 3 % by weight of bio-polymer. In accordance with the present invention, the components of the hydrogel are substantially chemically cross-linked so as to be non-extractable.

The relative amounts of each polymer to be included in the hydrogel similarly will be dependent on the type of synthetic polymer and bio-polymer being used and upon the intended application for the hydrogel. One skilled in the art will appreciate that the relative amounts of water, bio-polymer and synthetic polymer ratios will influence the final gel properties in various ways, for example, high quantities of water will produce a very soft hydrogel; high quantities of bio-polymer will produce a very stiff hydrogel and high concentrations of synthetic polymer will produce an opaque hydrogel. In accordance with the present invention, the weight per weight ratio of synthetic polymer : bio-polymer is between about 1 : 0.5 and about 1 : 20. In one embodiment of the present invention the ratio of synthetic polymer : bio-polymer is between 1 : 1 and 1 : 3.

2.3 Incorporation of Bioactive Agents into the Bio-synthetic Matrix

Bioactive agents can be incorporated into the matrix either by covalent attachment (or "grafting") to the synthetic polymer through the pendant reactive groups, or by encapsulation within the matrix. Examples of bioactive agents that may be covalently attached to the synthetic polymer component of the matrix are given above. For covalent attachment of a bioactive agent, the synthetic polymer is first reacted with the bioactive agent and then subsequently cross-linked to the bio-polymer as described above. Reaction of the bioactive agent with the synthetic polymer can be conducted under standard conditions, for example by mixing the bioactive agent and the synthetic polymer together in a non-aqueous solvent, such as N,N'-dimethyl formamide. The use of a non-aqueous solvent avoids hydrolysis of the reactive groups during incorporation of the bioactive agent. Alternatively, the reaction may be conducted as described above for the cross-linking reaction.

Bioactive agents which are not suitable for grafting to the polymer, for example, those that do not contain primary amino or free thiol groups for reaction with succinimidyl groups in the synthetic polymer, or which cannot be derivatised to provide such groups, can be entrapped in the final matrix. Examples of bioactive agents which may be entrapped in the matrix include, but are not limited to, certain pharmaceutical drugs, diagnostic agents, viral vectors, nucleic acids and the like. For entrapment, the bioactive agent is added to a solution of the synthetic polymer in an appropriate solvent prior to mixture of the synthetic polymer and the bio-polymer to form a cross-linked hydrogel. Alternatively, the bioactive agent can be added to a solution containing both the synthetic and bio-polymers prior to the cross-linking step. The bioactive agent is mixed into the polymer solution such that it is substantially uniformly dispersed therein, and the hydrogel is subsequently formed as described above. Appropriate solvents for use with the bioactive agent will be dependent on the properties of the agent and can be readily determined by one skilled in the art.

2.4 Entrapment of Cells in the Bio-synthetic Matrix

The bio-synthetic matrix according to the present invention may also comprise cells entrapped therein and permit delivery of the cells to a tissue or organ *in vivo*. A variety of different cell types may be delivered using the bio-synthetic matrix, for example, myocytes, ocular cells (*e.g.* from the different corneal layers), adipocytes, fibromyoblasts, ectodermal cells, muscle cells, osteoblasts (*i.e.* bone cells), chondrocytes (*i.e.* cartilage cells), endothelial cells, fibroblasts, pancreatic cells, hepatocytes, bile duct cells, bone marrow cells, neural cells, genitourinary cells (including nephritic cells), or combinations thereof. The matrix may thus be used to deliver totipotent stem cells, pluripotent progenitor cells or re-programmed (dedifferentiated) cells to an *in vivo* site such that cells of the same type as the tissue can be produced. For example, mesenchymal stem cells, which are undifferentiated, can be delivered in the matrix. Examples of mesenchymal stem cells include those which can diversify to produce osteoblasts (to generate new bone tissue), chondrocytes (to generate new cartilaginous tissue), and fibroblasts (to produce new connective tissue).

Cells can be readily entrapped in the final matrix by addition of the cells to a solution of the synthetic polymer prior to admixture with the bio-polymer to form a cross-linked hydrogel. Alternatively, the cells can be added to a solution containing both the synthetic and bio-polymers prior to the cross-linking step. The synthetic polymer may be reacted with a bioactive agent prior to admixture with the cells if desired. Typically, for the encapsulation of cells in the matrix, the various components (cells, synthetic polymer and bio-polymer) are dispersed in an aqueous medium, such as a cell culture medium or a diluted or modified version thereof. The cell suspension is mixed gently into the polymer solution until the cells are substantially uniformly dispersed in the solution, then the hydrogel is formed as described above.

3. *TESTING THE BIO-SYNTHETIC MATRIX*

In accordance with the present invention, the bio-synthetic matrix comprises a hydrogel with or without added bioactive agents and/or encapsulated cells. In order to be suitable for *in vivo* implantation for tissue engineering purposes, the bio-synthetic matrix must maintain its form at physiological temperatures, be adequately robust, and support the growth of cells. It may also be desirable for the matrix to support the growth of nerves. It will be readily appreciated that for certain specialised applications, the matrix may require other characteristics. For example, for surgical purposes, the matrix may need to be relatively flexible as well as strong enough to support surgical manipulation with suture thread and needle, and for ophthalmic applications, such as cornea repair or replacement, the optical clarity of the matrix will be important.

3.1 Physical / Chemical testing

The ability of the bio-synthetic matrix to withstand shearing forces can be roughly determined by applying forces in opposite directions to the specimen using two pairs of forceps. Quantitative characterisation can be achieved, for example, through the use of suture pull-out measurements on moulded matrix samples. For example, for matrix that has been moulded in the shape and thickness of a human cornea, two diametrically opposed sutures can be inserted into the matrix, as would be required for the first step in ocular implantation. The two sutures can then be pulled apart at 10 mm/min on a suitable instrument, such as an Instron Tensile Tester. Strength at

rupture of the matrix is calculated, together with elongation at break and elastic modulus. Optical transmission and light scatter can also be measured for matrices intended for ophthalmic applications using a custom-built instrument that measures both transmission and scatter [see, for example, Priest and Munger, *Invest. Ophthalmol. Vis. Sci.* 39: S352 – S361 (1998)].

3.2 In vitro *Testing*

It will be readily appreciated that the bio-synthetic matrix must be non-cytotoxic in order to be suitable for *in vivo* use. The cytotoxicity of the bio-synthetic matrix and its ability to support cell growth can be assessed *in vitro* using standard techniques.

For example, cells from an appropriate cell line, such as human endothelial cells, can be seeded either directly onto the matrix or onto an appropriate material surrounding the matrix. After growth in the presence of a suitable culture medium for an appropriate length of time, histological examination of the matrix can be conducted to determine whether the cells have grown over the surface of and/or into the matrix. Alternatively, varying concentrations of the matrix can be dissolved in culture medium and the resulting solution can be tested for its ability to support cell growth.

The ability of the matrix to support in-growth of nerve cells can also be assessed *in vitro*. For example, a nerve source, such as dorsal root ganglia, can be embedded into an appropriate material surrounding the matrix. Cells from an appropriate cell line can then be seeded either directly onto the matrix or onto an appropriate material surrounding the matrix and the matrix can be incubated in the presence of a suitable culture medium for an appropriate length of time. Examination of the matrix, directly and / or in the presence of a nerve-specific marker, for example by immunofluorescence using a nerve-specific fluorescent marker, for nerve growth will indicate the ability of the matrix to support neural in-growth.

3.3 In vivo *Testing*

In order to assess the biocompatibility of the bio-synthetic matrix and its ability to support cell growth *in vivo*, the matrix can be implanted into an appropriate animal model. At various stages

post-implantation, biopsies can be taken to assess cell growth over the surface of and/or into the implant. Histological examination can also be used to determine whether nerve in-growth has occurred and whether inflammatory or immune cells are present at the site of the implant. Measurement of the nerve action potentials using standard techniques will provide an indication of whether the nerves are functional.

4. APPLICATIONS

The present invention provides a functional bio-synthetic matrix which is robust, biocompatible and non-cytotoxic and therefore suitable for use as a scaffold to allow tissue regeneration *in vivo*. For example, the bio-synthetic matrix can be used for implantation into a patient to replace tissue that has been damaged or removed, for wound coverage, as a tissue sealant or adhesive, as a skin substitute or as a cornea substitute. The matrix can be moulded into an appropriate shape prior to implantation, for example it can be pre-formed to fill the space left by damaged or removed tissue. Alternatively, when used as an implant, the matrix may be allowed to form *in situ* by injecting the components into the damaged tissue and allowing the polymers to cross-link and gel at physiological temperature. In one embodiment of the present invention, the matrix is pre-formed. In a related embodiment the matrix is pre-formed as a full thickness artificial cornea or as a partial thickness matrix suitable for a cornea veneer.

The bio-synthetic matrix can be used alone and as such will support the in-growth of new cells *in situ*. Alternatively, the matrix can be seeded with cells prior to implantation and will support the outgrowth of these cells *in vivo* to repair and/or replace the surrounding tissue. It is contemplated that the cells may be derived from the patient, or they may be allogeneic or xenogenic in origin. For example, cells can be harvested from a patient (prior to, or during, surgery to repair the tissue) and processed under sterile conditions to provide a specific cell type such as pluripotent cells, stem cells or precursor cells. These cells can then be seeded into the matrix, as described above and the matrix can be subsequently implanted into the patient.

The matrix can also be used to coat surgical implants to help seal tissues or to help adhere implants to tissue surfaces, for example, through the formation of cross-links between unreacted

succinimidyl groups on the synthetic polymer component of the hydrogel and primary amino or thiol groups present in the tissue. For example, a layer of the matrix may be used to patch perforations in the corneas, or to catheters or breast implants to reduce fibrosis, or applied to vascular grafts or stents to minimise blood or serosal fluid leakage, to artificial patches or meshes to minimise fibrosis and help adhesion of the implants to tissue surfaces.

The matrix may also be used to deliver a bioactive agent to a patient. The bioactive agent can be delivered together with the synthetic and bio-polymers such that the matrix comprising the bioactive agent can form *in situ*, or the matrix comprising the bioactive agent can be pre-formed and implanted. Once within the body, the bioactive agent may be released from the matrix through diffusion-controlled processes or, if the bioactive agent is covalently bound to the matrix, it may be enzymatically cleaved from the matrix and subsequently released by diffusion-controlled processes or it may exert its effects from within the matrix.

In one embodiment of the present invention, the bio-synthetic matrix is used as an artificial cornea. For this application, the hydrogel is designed to have a high optical transmission and low light scattering. For example, hydrogels comprising a synthetic pNiPAAm-co-AAc-co-N-acryloxysuccinimide terpolymer cross-linked to collagen have high optical transmission, very low light scattering and are capable of remaining clear up to 55°C. The artificial cornea can be prepared by admixture of the synthetic and bio-polymers and injection of the resultant mixture into a suitable mould. After cross-linking at 4°C, the incubation temperature can then be raised to about 37°C to allow for the formation of the final hydrogel. The artificial cornea thus formed can be washed extensively in to remove N-hydroxysuccinimide produced by the cross-linking reaction and by hydrolytic termination of any unreacted cross-linking groups remaining in the matrix prior to use.

5. KITS

The present invention also contemplates kits comprising the bio-synthetic matrix. The kits may comprise a "ready-made" form of the matrix or they may comprise the individual components required to make the matrix (*i.e.* the synthetic polymer, with or without attached bioactive agents,

and the bio-polymer) in appropriate proportions. The kits may further comprise instructions for use, one or more suitable solvents, one or more instruments for assisting with the injection or placement of the final matrix composition within the body of an animal (such as a syringe, pipette, forceps, eye dropper or similar medically approved delivery vehicle), or a combination thereof. Individual components of the kit may be packaged in separate containers. The kit may further comprise a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of biological products, which notice reflects approval by the agency of the manufacture, use or sale for human or animal administration.

To gain a better understanding of the invention described herein, the following examples are set forth. It should be understood that these examples are for illustrative purposes only. Therefore, they should not limit the scope of this invention in any way.

EXAMPLES

Abbreviations

RTT:	rat-tail tendon
ddH_2O:	distilled, de-ionised water
PBS:	phosphate buffered saline
D-PBS:	Dulbecco's phosphate buffered saline
AIBN:	azobis-isobutyronitrile
NiPAAm:	N-isopropylacrylamide
pNiPAAm:	poly(N-iso-propylacrylamide)
AAc:	acrylic acid
ASI:	N-acryloxysuccinimide
pNIPAAm-co-AAc:	copolymer of NiPAAm and AAc
poly(NiPAAm-co-AAc-co-ASI):	terpolymer of N-isopropylacrylamide, (NIPAAM), acrylic acid (AAc) and N-acryloxysuccinimide (ASI)
GPC:	gel permeation chromatography

All gel matrices described in the Examples set out below used sterile collagen I, such as telocollagen (rat-tail tendon, RTT) or atelocollagen (bovine or porcine), which can be prepared in the laboratory or more conveniently is available commercially (for example, from Becton Dickinson at a concentration of 3.0-3.5 mg/ml in 0.02N acetic acid). Such collagens can be stored for many months at 4°C. In addition, such collagen solutions may be carefully concentrated to give optically clear, very viscous solutions of 3 – 6 wt/vol % collagen, suitable for preparing more robust matrices.

Collagen solutions are adjusted to physiological conditions, *i.e.* saline ionic strength and pH 7.2 – 7.4, through the use of aqueous sodium hydroxide in the presence of phosphate buffered saline (PBS). PBS, which is free of amino acids and other nutrients, was used to avoid depletion of cross-linking reactivity by side reactions with $-NH_2$ containing molecules, so allowing the use of the minimum concentration of cross-linking groups and minimising any risk of cell toxicity.

PNiPAAm homopolymer powder is available commercially (for example, from Polyscience). All other polymers were synthesized as outlined below.

EXAMPLE 1: PREPARATION OF A pNIPAAm-COLLAGEN HYDROGEL

A 1 wt/vol% solution of pNiPAAm homopolymer in ddH_2O was sterilised by autoclaving. This solution was mixed with sterile RTT collagen solution [3.0-3.5 mg/ml (w/v) in acetic acid (0.02N in water] (1:1 vol/vol) in a sterile test tube at 4°C by syringe pumping to give complete mixing without bubble formation. Cold mixing avoids any premature gelification or fibrilogenesis of the collagen. The collagen-pNiPAAm was then poured over a plastic dish (untreated culture dish) or a mould (*e.g.* contact lens mould) and left to air-dry under sterile conditions in a laminar flow hood for at least 2-3 days at room temperature. After drying to constant weight (~7 % water residue), the formed matrix was removed from the mould. Removal of the matrix from the mould is facilitated by soaking the mould in a sterile PBS at room temperature. Continued soaking of the free sample in this solution gives a gel at physiological pH and ionic strength, suitable for cell growth and *in vivo* animal testing.

EXAMPLE 2: PREPARATION OF A SYNTHETIC TERPOLYMER

A collagen-reactive terpolymer, poly(NiPAAm-co-AAc-co-ASI) (Figure 1), was synthesised by co-polymerising the three monomers: N-isopropylacrylamide, (NiPAAm, 0.85 mole), acrylic acid (Aac, 0.10 mole) and N-acryloxysuccinimide (ASI, 0.05 mole). The feed molar ratio was 85:10:5 (NiPAAm: AAc: ASI), the free-radical initiator AIBN (0.007 mole/mole of total monomers) and the solvent, dioxane (100 ml), nitrogen purged before adding AIBN. The reaction proceeded for 24 h at 65°C.

After purification by repeated precipitation to remove traces of homopolymer, the composition of the synthesised terpolymer (82% yield) was found to be 84.2:9.8:6.0 (molar ratio) by proton NMR. The M_n of the terpolymer is 5.6×10^4 Da by aqueous GPC.

A solution of 2 mg/ml of the terpolymer in D-PBS remained clear even up to 55°C. A solution of 10 mg/ml in D-PBS became only slightly cloudy at 43°C. Failure to remove homopolymer formed in the batch polymerisation reaction (due to the NiPAAm reactivity coefficient being greater than that of AAc or ASI) from the terpolymer gave aqueous solutions and hydrogels which cloud at ~32°C and above.

EXAMPLE 3: PREPARATION OF A SYNTHETIC POLYMER COMPRISING A BIOACTIVE AGENT

A terpolymer, containing the pentapeptide YIGSR (a nerve cell attachment motif), was synthesised by mixing the terpolymer prepared in Example 2 (1.0 g) with 2.8μg of laminin pentapeptide (YIGSR, from Novabiochem) in N,N-dimethyl formamide. After reaction for 48 h at room temperature, the polymer product was precipitated out from diethyl ether and then vacuum dried. It was assumed that all the YIGSR pentapeptide was attached to the reactive terpolymer because the pendant, reactive ASI groups are in large excess. ASI groups remaining after reaction with the pentapeptide are available for subsequent reaction with collagen.

EXAMPLE 4: PREPARATION OF A COLLAGEN-TERPOLYMER HYDROGEL

A cross-linked, terpolymer-collagen hydrogel was made by mixing neutralised 4% bovine collagen (1.2 ml) with the terpolymer prepared in Example 2 [0.34ml (100 mg/ml in D-PBS)] by syringe mixing at 4°C. After careful syringe pumping to produce a homogeneous, bubble-free solution, aliquots were injected into plastic, contact lens moulds and incubated at room temperature for 24 hours to allow reaction of the collagen $-NH_2$ groups with ASI groups as well as the slower hydrolysis of residual ASI groups to AAc groups. The moulded samples were then incubated at 37°C for 24 hours in 100% humidity environment, to give a final hydrogel. The hydrogel contained 95.4 ± 0.1% water, 2.3% collagen and 1.6% terpolymer. Matrices were moulded to have a final thickness between either 150 - 200 μm or 500 – 600 μm. Each resulting hydrogel matrix was removed from its mould under PBS solution and subsequently immersed in PBS containing 1% chloroform and 0.5% glycine. This wash step removed N-hydroxysuccinimide produced in the cross-linking reaction and terminated any unreacted ASI groups in the matrix, by conversion to acrylic acid groups.

Succinimide residues left in the gels prepared from collagen and terpolymer were below the IR detection limit after washing.

EXAMPLE 5: PREPARATION OF A HYDROGEL COMPRISING A BIOACTIVE AGENT

Cross-linked hydrogels of collagen-terpolymer comprising YIGSR cell adhesion factor were prepared by thoroughly mixing viscous, neutralised 4% bovine collagen (1.2 ml) with terpolymer to which laminin pentapeptide (YIGSR) was covalently attached (from Example 3; 0.34ml, 100 mg/ml) at 4°C, following the procedure described in Example 4.

EXAMPLE 6: COMPARISON OF THE PHYSICAL PROPERTIES OF HYDROGEL MATRICES

Collagen thermogels (prepared at 37°C, without any chemical cross-linker added) are frail and readily collapse and break and are obviously opaque (see Figure 10).

The following properties of the hydrogels prepared as described in Examples 4 and 5 indicate that they are cross-linked:

- water insoluble,
- strong enough to support surgical manipulation with suture thread and needle
- relatively flexible in handling
- demonstrate an increase in stress at failure and apparent modulus during tensile testing by over x2 on going from $-NH_2$/ASI equivalent ratio of 0.5 to 1.5.

Quantitative characterisation of the hydrogels came from the use of suture pull-out measurements on samples moulded into the shape and thickness of a human cornea. This involved insertion of two diametrically opposed sutures, as required for the first step in ocular implantation, and pulling these two sutures apart at 10 mm/min on an Instron Tensile Tester, a procedure that is well established for the evaluation of heart valve components. Strength at rupture of the gel is calculated, together with elongation at break and elastic modulus.

The hydrogels prepared as described in Examples 4 and 5 have high optical transmission and very low light scattering, comparable to the human cornea, as measured with a custom-built instrument that measures transmission and scatter [Priest and Munger, Invest. Ophthalmol. Vis. Sci. 39: S352 – S361 (1998)]. In contrast, collagen- pNIPAAm homopolymer gels (as described in Example 1; 1.0 : 0.7 to 1.0 : 2.0 wt/wt) were opaque at 37°C. In addition, the pNiPAAm homopolymer in gels from Example 1 extracts out into aqueous media, including physiological liquids.

Figure 10 shows (A) the structure of the terpolymer. The increased transparency in the corneal scaffold composed of the collagen terpolymer is also shown in Figure 10: (B) thermogelled collagen only and (C) collagen-terpolymer.

EXAMPLE 7: **IN VIVO *TESTING OF VARIOUS BIO-SYNTHETIC MATRICES***

Hydrogels formed as described in Examples 1, 4 and 5 were moulded to form artificial corneas and implanted into the eyes of pigs (Figure 2).

As *in vivo* corneal implants, the gels from Example 1 exude white residue when implanted in pigs' eyes.

The hydrogel prepared from 4% collagen and pentapeptide-terpolymer as described in Example 5 demonstrated good biocompatibility as did the collagen-terpolymer hydrogel prepared as described in Example 4. More rapid, complete epithelial cell overgrowth and formation of multiple layers occurred when the former hydrogel was used, as compared to collagen-terpolymer hydrogel which showed slower, less contiguous, epithelial cell growth, without formation of multiple layers.

In vivo, confocal microscope images of full thickness hydrogel prepared from collagen and the pentapeptide-terpolymer (from Example 5; final concentration: collagen 2.3 wt %; terpolymer + pentapeptide 1.6 wt %) and implanted into a pig's eye showed that epithelium cells grew over this matrix and stratified. A basement membrane was regenerated and hemidesmosomes, indicating a stably anchored epithelium, were present. Stromal cells were found to spread inside the matrix after only three weeks. The implants became touch sensitive within 3 weeks of implantation (Cochet-Bonnet Aesthesiometer) indicating functional nerve in-growth. Nerve in-growth was also observed directly by confocal microscopy and histology. No clinical signs of adverse inflammation or immune reaction were observed over an 8 week period following implantation. See Figures 3 – 8.

Figure 9 shows (A) a section through the pig cornea at 3 weeks post-implantation, stained with picro-sirius red, which demonstrates the stromal-implant interface (arrowheads). The implant surface has been re-covered by a stratified epithelium. (B) a similar section at 8 weeks post-implantation. Stromal cells have moved into the implant and the implant appears to have been replaced by tissue sub-epithelially (arrows). (C) a higher magnification of the epithelium (H & E stained) showing the regenerated basement membrane (arrow). (D) a corresponding section stained with anti-type VII collagen antibody that recognizes hemidesmosomes attached to the basement membrane (arrow). (E) the hemidesmosomes (arrows) attached to the underlying basement membranes are clearly visualized by transimission electron microscopy (TEM). (F) a

flat mount of the pig cornea showing nerves (arrowheads) within the implant, stained with an anti-neurofilament antibody.

Whole mount confocal microscopic images of pigs corneas at 6 weeks post-operation showing a regenerated corneal epithelium (Figure 10D) and basement membrane (Figure 10E) on the surface of the implant. *In vitro* nerve growth patterns within the collagen-terpolymer composite are shown in Figure 10F and within the underlying host stroma in Figure 10G. Figure 10H shows in-growing stromal cells.

EXAMPLE 8: NEURAL IN-GROWTH INTO COLLAGEN-TERPOLYMER MATRICES IN RODENT BRAIN

Following euthanasia, the whole brain of each mouse or rat used was excised and placed within a sterotaxic frame. Either two microlitres (2 ml) or three microlitres (3 ml) of hydrogel containing collagen, terpolymer-pentapeptide at either 0.33% collagen - 0.23% terpolymer or 0.63% collagen-0.44% terpolymer was injected over a period of 6 to 10 min, respectively, into each individual mouse brain, at the following coordinates: 0.3 mm from bregma, 3.0 mm deep and 2.0 mm from the midline. For rats, four to six microlitres of hydrogel was injected over 10 min. into each brain, at 0.7-0.8 mm from bregma, 6 mm deep and 4 mm from the midline. The hydrogel samples were mixed with Coomassie blue dye for visualization.

Results indicate successful direct, precise delivery of small amount of the hydrogel into the stratum of the brain, in these samples. This suggests that it is possible to use the hydrogel as a delivery system for cells or drugs into specific locations at very small volumes.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A terpolymer comprising N-isopropylacrylamide, acrylic acid and N-acryloxysuccinimide.

2. A bio-synthetic matrix comprising:
   (a) a terpolymer comprising N-isopropylacrylamide, acrylic acid and N-acryloxysuccinimide, and
   (b) a biopolymer,
   wherein said terpolymer and said biopolymer are cross-linked.

3. Use of a bio-synthetic matrix as a scaffold to support tissue regeneration *in vivo*, wherein said bio-synthetic matrix comprises:
   (a) a terpolymer comprising N-isopropylacrylamide, acrylic acid and N-acryloxysuccinimide, and
   (b) a biopolymer,
   and wherein said terpolymer and said biopolymer are cross-linked.

4. Use of a bio-synthetic matrix to deliver one or more bioactive agents to a tissue or organ in a mammal, wherein said bio-synthetic matrix comprises:
   (a) a terpolymer comprising N-isopropylacrylamide, acrylic acid and N-acryloxysuccinimide, and
   (b) a biopolymer,
   and wherein said terpolymer and said biopolymer are cross-linked.

5. The terpolymer according to claim 1, the bio-synthetic matrix according to claim 2, or the use according to claim 3 or 4, wherein said biopolymer is collagen.

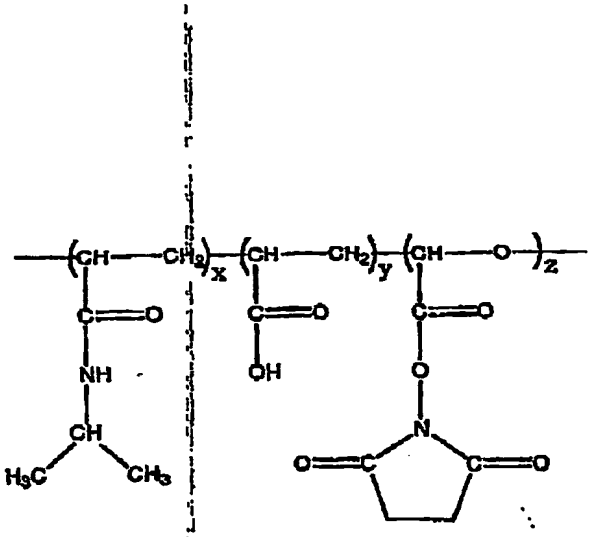


Figure 1

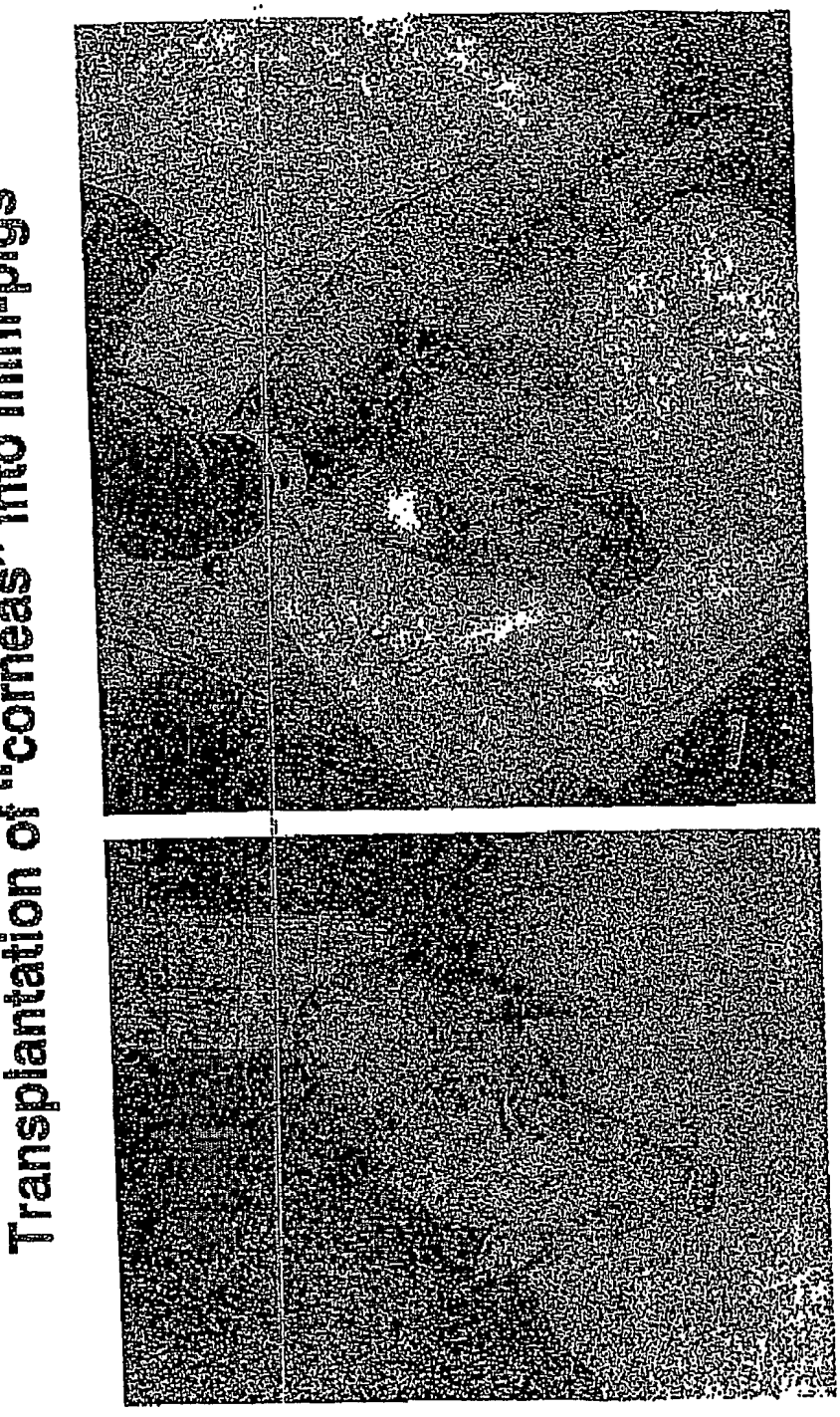


Figure 2

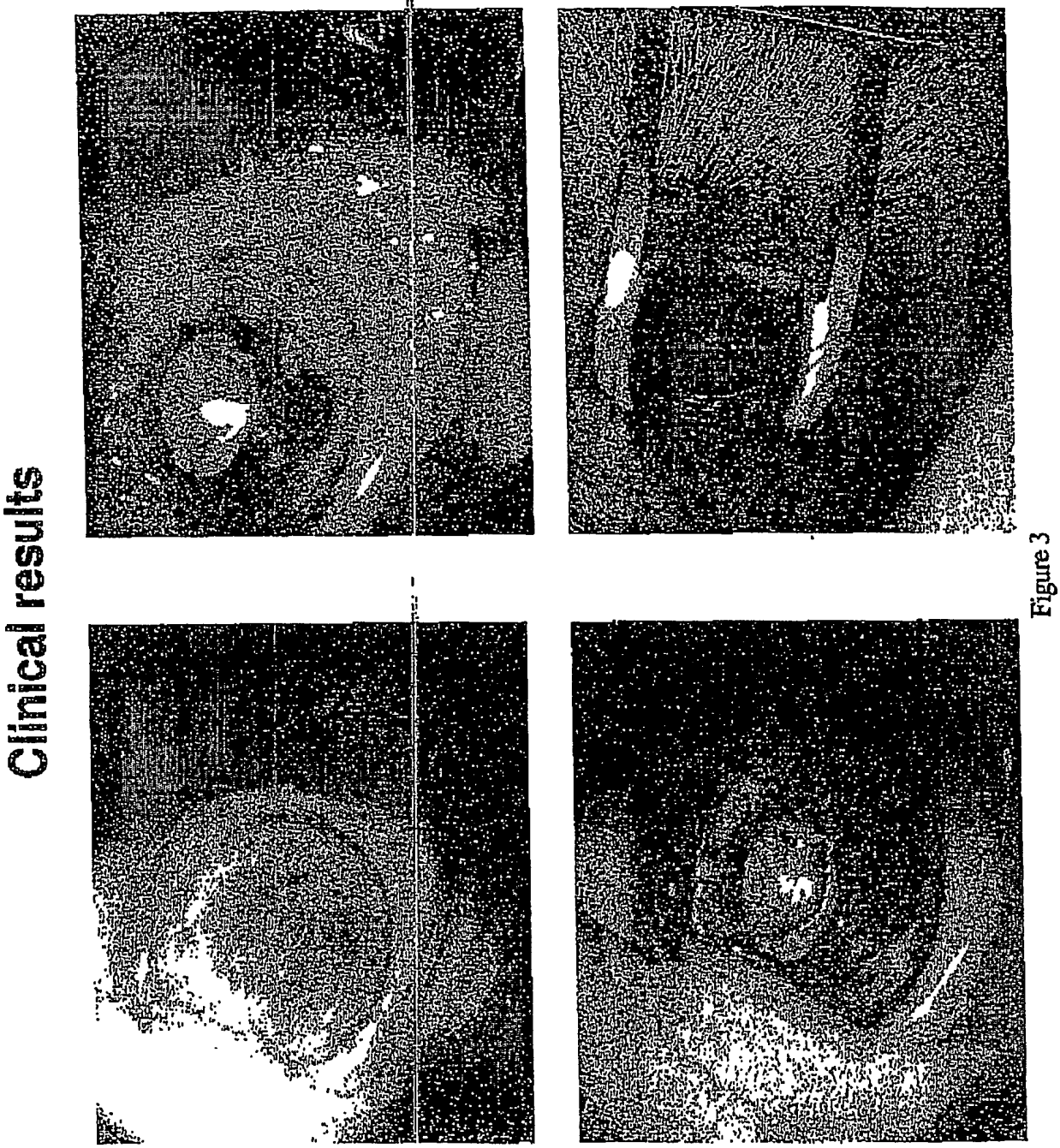


Figure 3

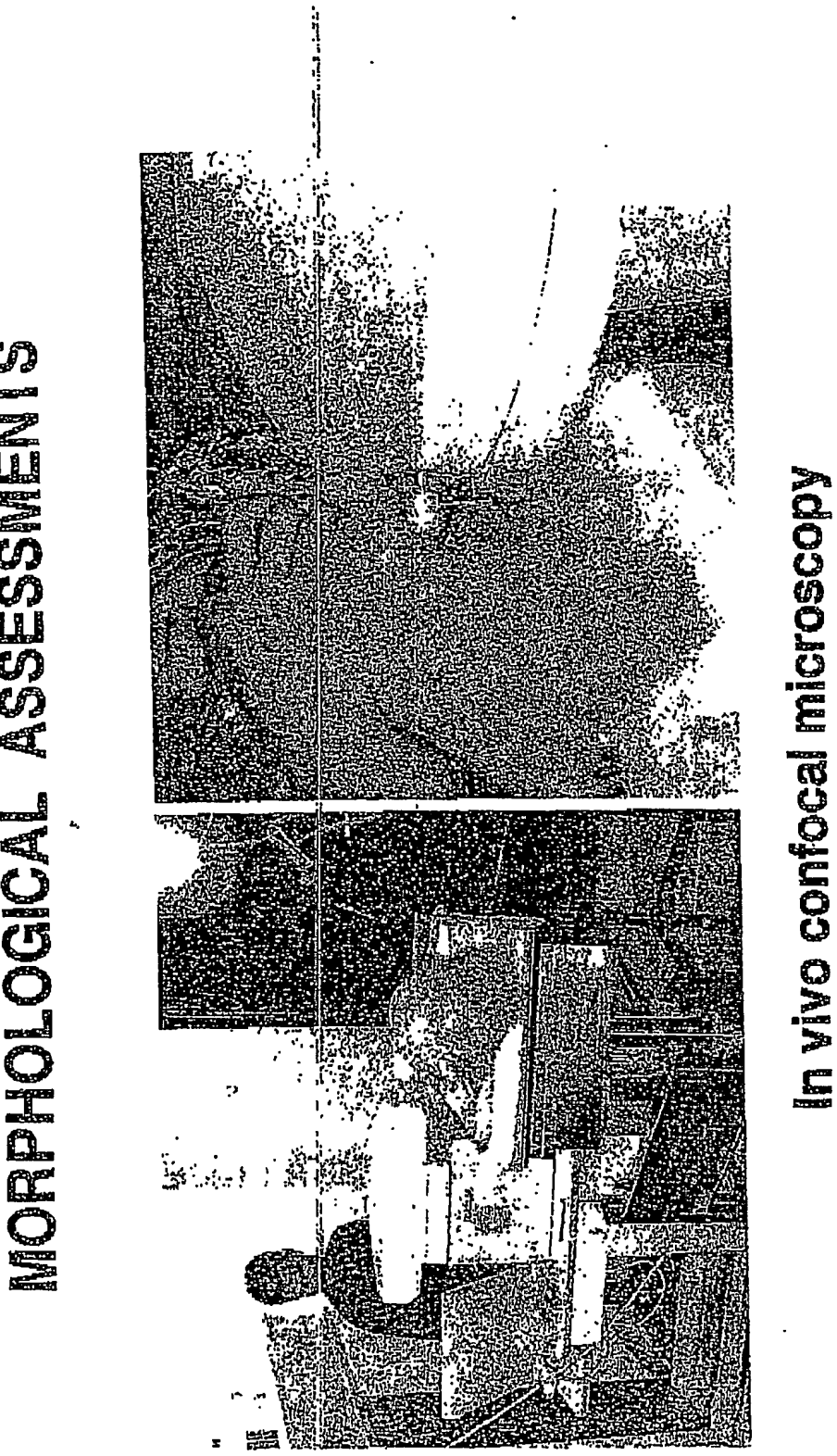


Figure 4

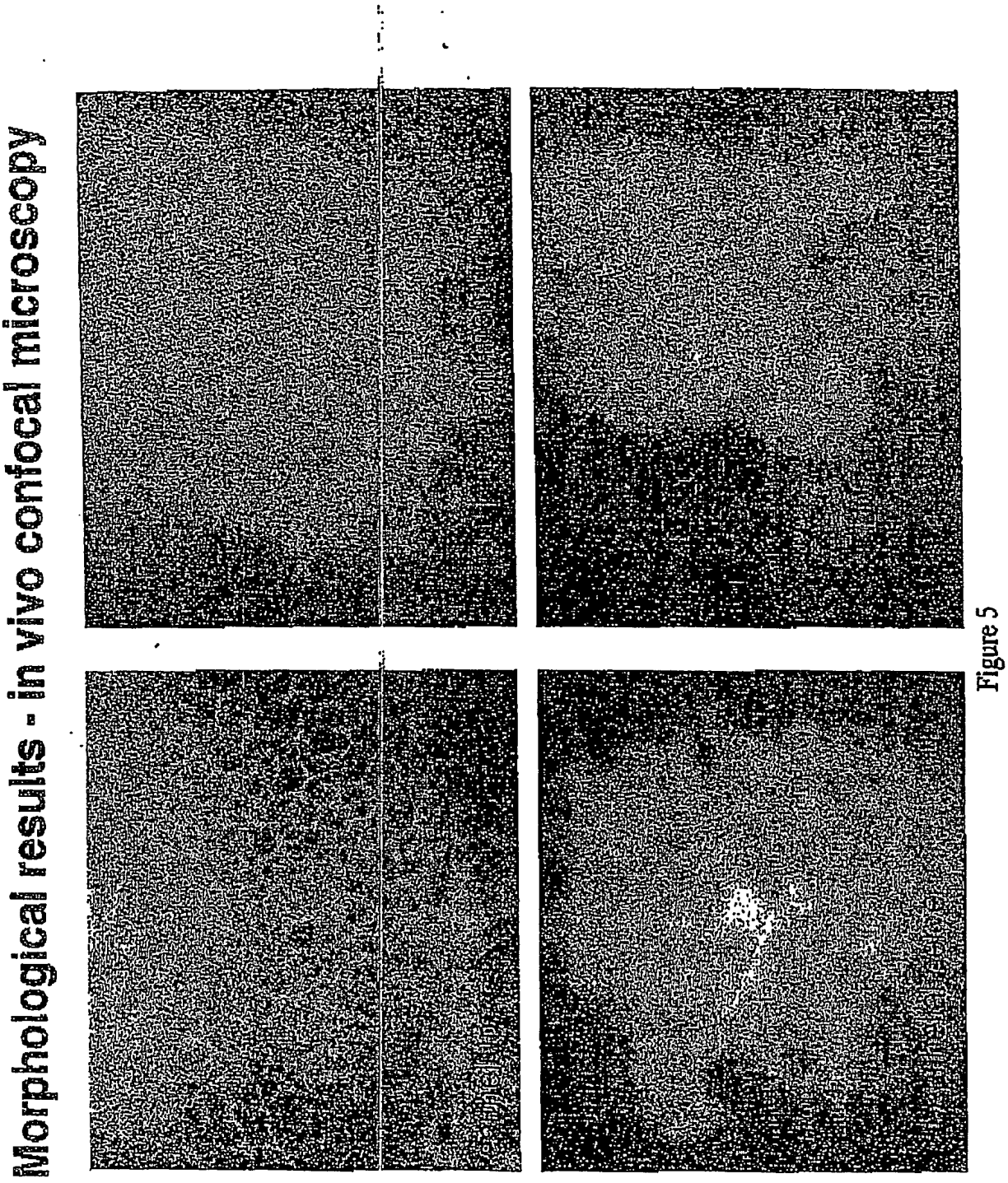


Figure 5

In vivo results (contd)

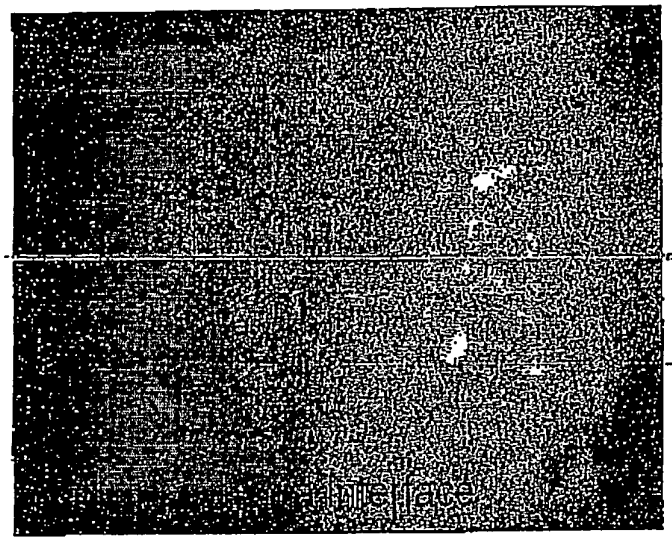
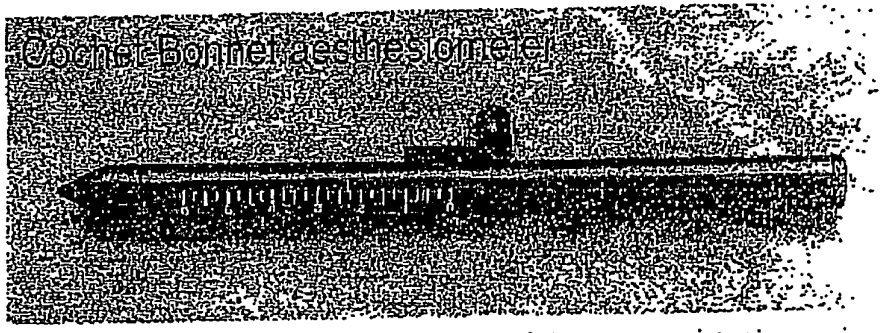
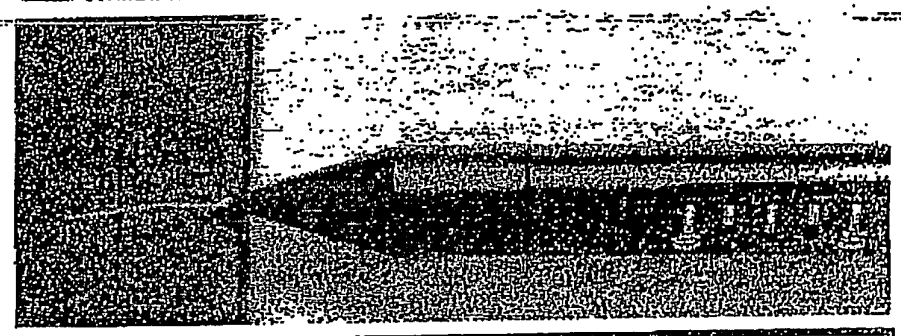
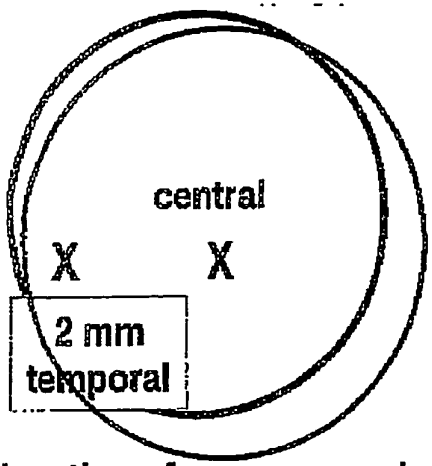


Figure 5 continued

Testing for Corneal Sensitivity







location of measurements

	preop	1d	7d	14d	21d
center:	+ + +	- - -	- - -	+ - -	+ + +
temporal:	+ + +	- - -	- - -	+ + -	+ + +

Figure 6


Morphological/Biochemical asessment
epi
polymer
stroma
H&E for cells
epi
polymer
Picro-sirius stain for collagen

Figure 7

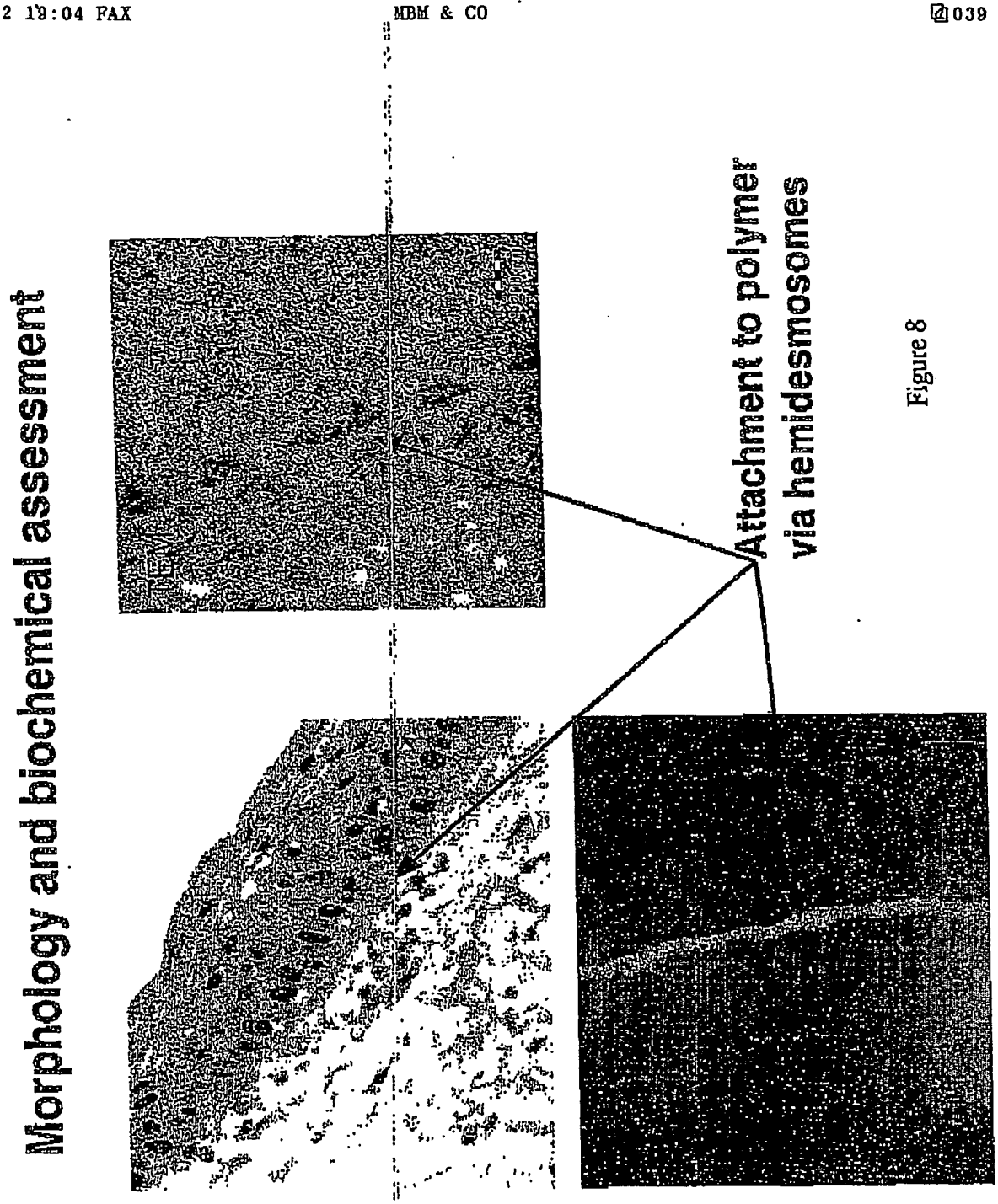

Morphology and biochemical assessment
Attachment to polymer via hemidesmosomes

Figure 8

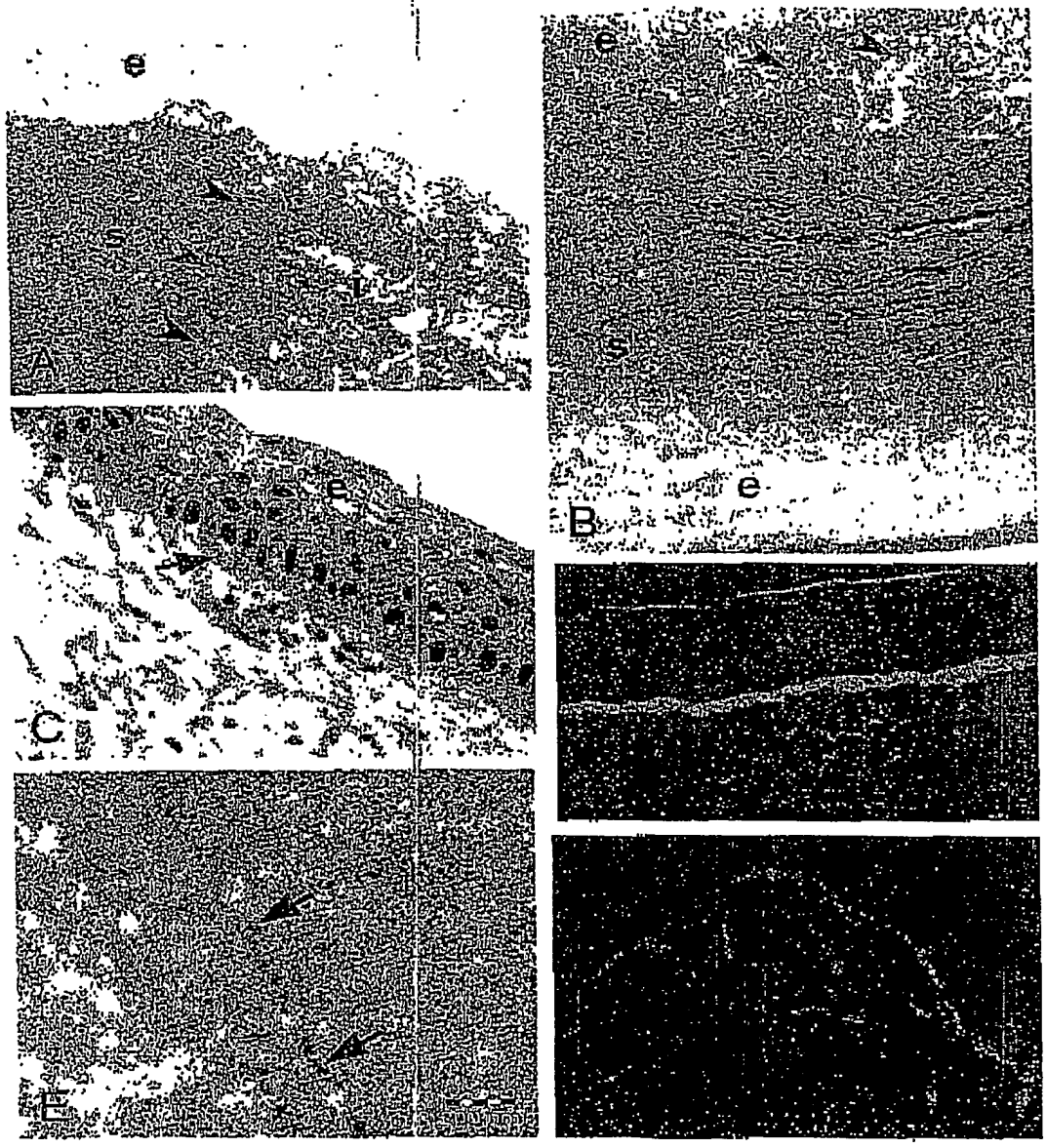


Figure 9

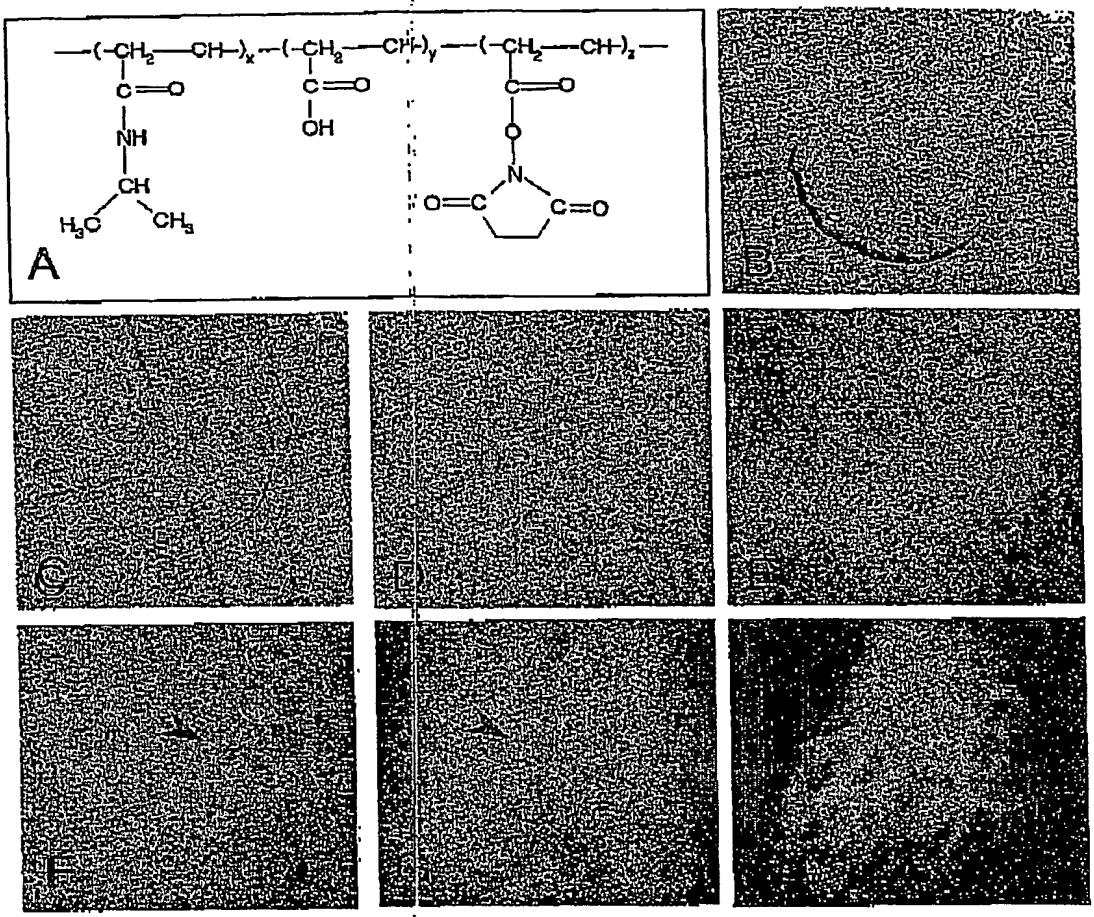


Figure 10

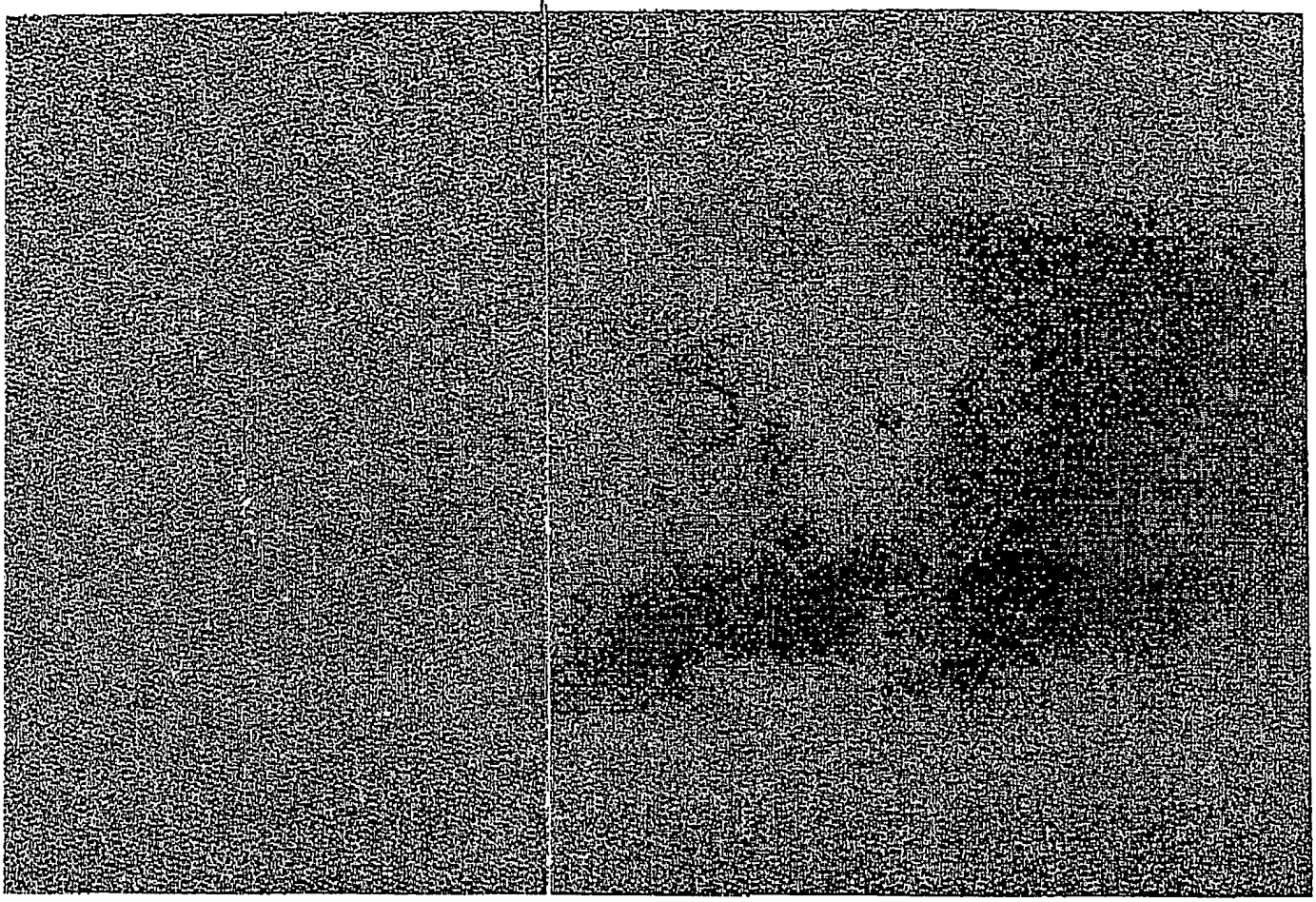


Figure 11

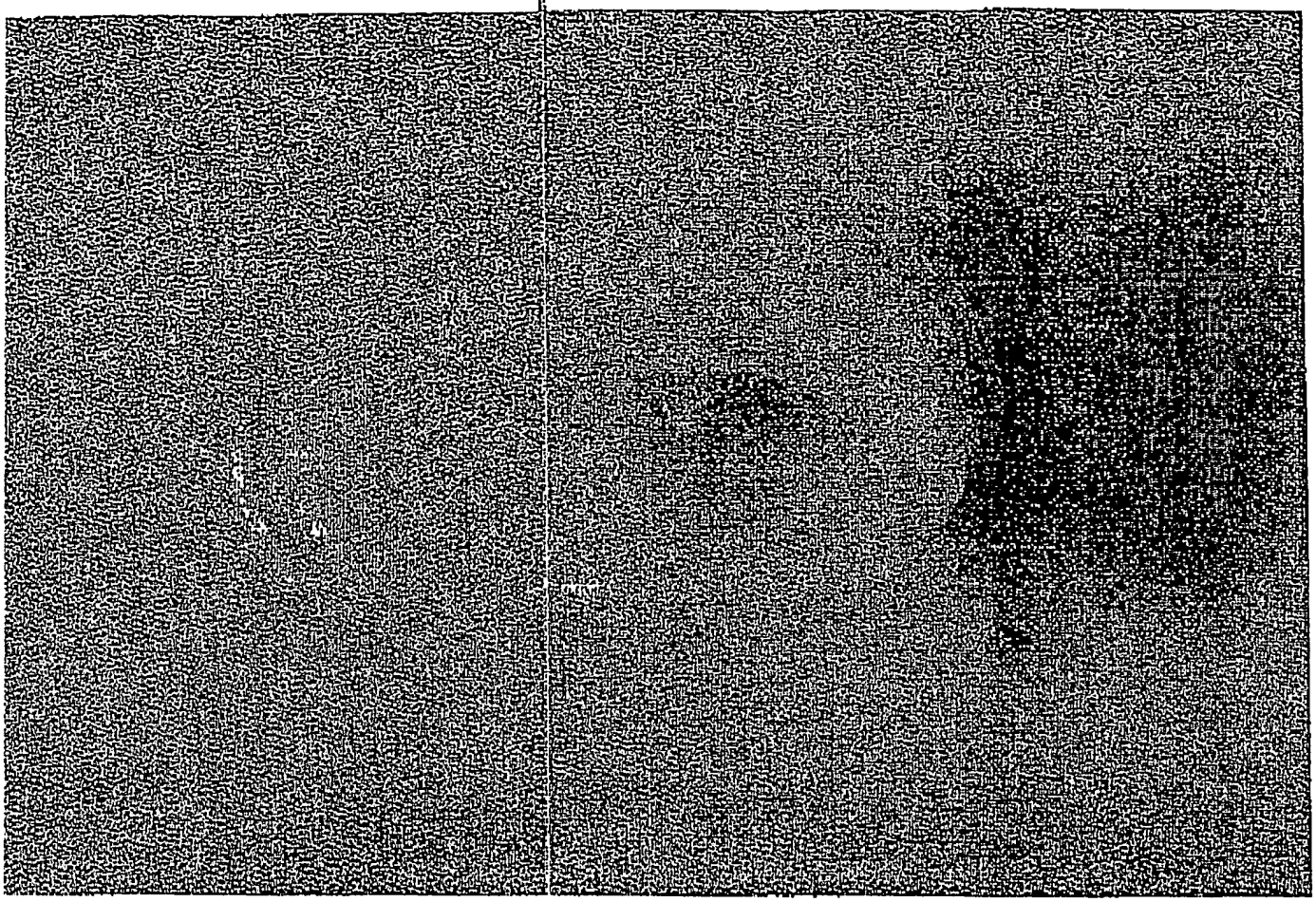


Figure 12